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Veterinary Immunology and Immunopathology
80 (2001) 93–109

Veterinary immunology and immunopathology

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Summary of workshop findings for porcine myelomonocytic markers

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Abstract

About 65 monoclonal antibodies (mAb) including 17 internal controls were analyzed for their ability to recognize and bind to various cells of the myelomonocytic lineage. Flow cytometry (FCM) utilizing both single and double staining, and immunoprecipitation (IP) assays were used in the analysis. About 38 of the mAb were reactive with myelomonocytic cells, resulting in nine clusters of interest. Although the exact identity of many of the molecules on the cells bound by the mAb remains undetermined, information obtained about the mAb analyzed in this workshop should be helpful in further identifying various populations of myelomonocytic cells and their stages of differentiation. Out of 12 mAbs with potential CD11 specificity, seven were assigned to three different swine specific alpha chains of the CD11/CD18 integrin heterodimer, the assignment of the remaining four was tentative. One antibody had a binding specificity consistent with SWC3 and one with SWC8. CD14 expression on pig cells was characterized with a panel of CD14-positive antibodies, two of these antibodies were assigned to swine CD14. Two antibodies were assigned to CD163. Further work is required to determine the antigens recognized by many of the other mAb.

Keywords: Pig; Cluster of differentiation (CD) antigens; Monoclonal antibodies (mAb); Myeloid cells; CD11; CD14; CD92; CD93; CD163; SWC3; SWC8

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0165-2427/01/$ – see front matter
PII: S0165-2427(01)00278-1

1. Introduction

Cells of myelomonocytic lineage play an important role in the immune response and induction of inflammation in swine. The identification of the various subsets of myelomonocytic cells is crucial for understanding swine diseases and the success of potential xenotransplantation. The First Swine Cluster of Differentiation (CD) Workshop analyzed the reactivity of a panel of 22 test monoclonal antibodies (mAb) against cells of myeloid origin. Based on the assay data, clustering analysis and inhibition studies, two mAb that recognized monocytic cells and neutrophils were assigned swine workshop cluster number 3 (SWC3) (Blecha et al., 1994). In the Second Swine CD Workshop 45 mAb were analyzed for their reactivity with myeloid cells. As a result, a new swine workshop cluster, SWC9 was identified. SWC9 was composed of two mAb that recognized an antigen on mature macrophages (Dominguez et al., 1998). The cross-reactivity of three anti-human CD14 mAbs with porcine myelomonocytic cells was also established in the second workshop. Continuing research outside the workshops has established that mAb G7 recognizes the porcine homologue to CD16 (Halloran et al., 1994) and mAb 2A10/11 is homologous to human CD163, thus relating to macrophage differentiation (Sanchez et al., 1999). In this workshop, a second antibody was assigned to wCD163, a third mAb to SWC8, an additional mAb to SWC3 and two mAbs to CD14. Three different CD11 antigens were identified, represented by a total number of seven antibodies. The cross-reactivity of the anti-human CD92 and CD93 mAbs was confirmed.

2. Methods

About 65 mAb identified in the first round analysis of the Third Swine CD Workshop were distributed to the participants of this paper. Table 1 summarizes the participants and their contributions. About 17 of the mAbs were included as internal controls and had been analyzed in previous Swine CD Workshops. Techniques used to analyze the mAb in this workshop included immunoprecipitation (IP) and flow cytometry (FCM), consisting of one and two colour analysis. For studies using peripheral blood (PB) cells, FCM gates were set, based on FS/SS characteristics, to measure binding of the mAb to lymphocytes, monocytes and granulocytes. Epitope mapping was conducted with mAb when feasible. A variety of procine myelomonocytic cell populations including peripheral blood (PB) cells, neutrophils, pulmonary alveolar macrophages (PAM), myeloid cells from the jejunal lamina propria (LP), monocyte derived macrophages (MDM) and bone marrow (BM) granulocytes and monocytes were used to evaluate the mAb. Macrophages were tested either unstimulated or following treatment with granulocyte, murine monocyte-colony stimulating factor (GM-CSF) or lipopolysaccharide (LPS). These data sets, together with data obtained in the first round analyses were used for information about cellular specificity as well as for statistical clustering, using the Leucocyte Typing Database IV software, developed for the Fourth International Workshop on Human Leucocyte Antigens (Gilks, 1990).

Table 1
Contribution of different laboratories to the myeloid section

Participants	Laboratory	Cells	Analysis
J. Dominguez, F. Alonso, A. Ezquerra	CIS-INIA. Madrid, Spain	PBMC[a], Gran[b], PAM[c]	FCM two-colour; Imm Ppt., epitope mapping
K. Haverson	Div. of Mol. and Cell Biol., University of Bristol, Langford, UK	GI lamina propria cells[d], PBL[e]	FCM, two-colour
J. Lunney	Immunol Dis Resist Lab, ARS, USDA, Beltsville, MD	PAM[c], Neutrophils	FCM
A. Summerfield, K.McCullough	Inst. Virol. Immun., Mittelhausern, Switzerland	BM[f], PBMC, mono[g]	FCM, three-colour
J. Sinkora	Institute of Gnotobiology, Navy Hradek, Czech Republic		MW analysis (MIL2)
E. Thacker	VMRI, Ames, IA	PBMC, MDM's[h] + GM-CSF[i] +/− LPS[j], PAM + GM-CSF +/− LPS	FCM, three-colour

[a] Cells used in the analysis: peripheral blood monocytes.
[b] Granulocytes.
[c] Pulmonary alveolar macrophages.
[d] Cells isolated from jejunal lamina propria, SWC3+, predominantly eosinophils (Haverson et al., 1994).
[e] Peripheral blood leucocytes.
[f] Bone marrow SWC3+ cells.
[g] Monocytes.
[h] Monocyte derived macrophages.
[i] Granulocyte–monocyte colony stimulating factor.
[j] Lipolysaccaride treated cells (10 g/ml).

3. Results

The binding reactivity of each mAb and cell type is summarized in Table 2. The statistical cluster analysis grouped the mAb into 18 clusters of at least two mAb (Fig. 1). Of these 18 clusters, nine clusters containing 38 mAb were determined to be reactive with myelomonocytic cells. The remaining antibodies either stained weakly or were non-specific for myeloid cells. The nine clusters that were further investigated included C2, C3, C4, C5, C6, C9, C11, C12 and C17.

3.1. Cluster C1

Two mAbs PGBL18A (no. 82) and PGBL1A (no. 83) formed this cluster. Although, these antibodies showed similar cell binding patterns and recognized most BM, peripheral myeloid cells and LP myeloid cells, they also seemed to recognize cells in the lymphocyte gated region and were not investigated further.

3.2. Cluster C2

MIL3 (no. 56) and JM7B6 (no. 141) made up this cluster. MIL3 (no. 56) along with MUC20A (not included in this workshop) had previously been analyzed in the adhesion molecule section of the Second Swine CD Workshop and assigned a SWC8 designation (Haverson et al., 1998). SWC8 is not found on freshly isolated monocytic cells, but is present on all BM, peripheral granulocytes and LP myeloid cells. However, SWC8 is not exclusively specific for myeloid cells, as MIL3 binds to B cells and $CD8^{high}$ T lymphocytes (Haverson et al., 1994) as well as non-leucocytic cells (Haverson et al., 1998). Neither of the SWC8 designated mAb bound fresh PAM. However, both MIL3 (no. 56) and JM7B6 (no. 141) bound to PAM following adherence of the cells to plastic and the intensity of staining increased with the addition of GM-CSF (data not shown). Although the mAb did not bind to monocytes, both mAb reacted with monocytes induced with GM-CSF to differentiate to MDMs. Cultured endothelial cells also showed variable expression of SWC8. These findings suggest that these mAb may recognize a molecule present on all granulocytes and are also associated with differentiation or activation of other cells, such as monocytes and macrophages. Functional assays need to be performed to further identify and characterize the molecule being recognized. Two colour staining with MIL3 (no. 56), MUC20A and JM7B6 (no. 141) also support the assignment of JM7B6 (no. 141) to SWC8 (Fig. 2) and competitive epitope analysis suggests that all three antibodies appear to recognize the same epitope (not shown).

3.3. Clusters C3 and C4

The mAbs in clusters 3–5 showed similar cell population specificity recognizing the majority of BM and peripheral granulocytes and monocytes as well as macrophages. They did not bind in significant levels to lymphocytes. Most mAb in clusters 3 and 4 appeared leucocyte specific, whereas many mAb in cluster 5 also recognized endothelial cell lines.

Table 2
Mean percentage binding of workshop mAb to the myelomoncytic cell targets

Workshop no.	mAb	Antigen assignment	PAM[a]	MDM[b]	Peripheral blood			GI cells[f]	BM		Workshop Ms[i]
					Grans[c]	Mono[d]	Lymph[e]		Mono[g]	Gran[h]	
14	TMG 6-5	CD11b	2.4	42.1	67.8	22.3	16.8	36.3	28	44	170
15	S-Hc13	CD11c	46.2		1.8	24.0	1.6	5.4	negative	negative	150
16	biG10/14	CD14	16.6		21.2	47.7	7.8	8.6	negative	negative	48–53
17	G7	CD16	93	16	96.6	79.5	17.3	13.4	>80	67	50–80
23	2 ZC115	CD32	23.8		3.2	10.5	4.6	1.2	negative	negative	40
24	Ber-MAC-DRC	CD35	1.7		3.3	18.3	3.6	2.9	negative	negative	50
25	G28.5	CD40	48.2		13.4	21.0	16.7	43.9	<10	<4	
35	AK7	CD49b	8.2		3.1	3.8	3.8	11.9	negative	negative	
42	CB3-1	CD79B	25.1		4.2	8.2	5.7	32.3	<10	<4	
44	VIM15	CDw92	71.4		47.9	45.1	4.8	1.8	negative	negative	70
45	VIMD2	CD93	18.1		51.8	45.7	20.6	4.6	negative	negative	110
46	K139 3E1	sIgG	93.7		2.01	15.0	18.2	7.3	<3	<3	24
50	74-22-15	SWC3a	99.6		98.5	54.5	4.8	50.8	>99	>99	
51	6F3	SWC3a	99.7		88.3	87.6	6.7	51.5	>99	>99	
56	MIL3	SWC8	25.6		92.5	6.6	39.6	40.6	<5	95	
57	C4	SWC9	85.9		6.2	8.7	5.9	6.5	<3	<3	130/>205
61	SwNL-517.2		79.4	42.7	5.8	58.2	3.7	3.7	20–40	<2	
62	SwNL-552.2		56.1		83.5	91.1	7.7	12.9	>90	40	
68	PT79A		62.8		2.2	3.7	31.8	8.3	<5	<3	3740
69	MUC127A		58.1		3.9	3.9	34.6	7.8	<10	<3	3740
70	MUC106A		6.9		86.4	10	32.8	10.3	<5	72	
71	PG104A		39.6		6.4	21.2	34.1	8.6	<15	<2	
72	RH1A		88.1	38.2	92.4	70.3	16.7	18.6	negative	negative	
73	MUC21A		36.4		42.1	44.3	8.9	35.6	40–60	52	
79	PG68A		9.6	41.3	91.5	26.9	3.4	11.2	15–30	52	
82	PGBL18A		66.4		95.8	55.1	20.4	40.3	>80	95	
83	PGBL1A		80.5		92.6	84.2	22.1	25.9	>80	78	
84	PGBL21A		62.4	38.3	98.9	79.4	9.4	28.5	>80	95	
85	CAM36A		26.9		89.3	74.2	45.2	9.3	9.3	44	

Table 2 (*Continued*)

Workshop no.	mAb	Antigen assignment	PAM[a]	MDM[b]	Peripheral blood			GI cells[f]	BM		Workshop Ms[i]
					Grans[c]	Mono[d]	Lymph[e]		Mono[g]	Gran[h]	
93	2C12/10		27.4	52.8	66.0	59.7	3.3	31	<90	<99	
94	5A6/8		92.2	42	3.1	22.5	7.2	6	<10	<3	20/29
98	4F10		32.2		89.3	60.7	13.9	4.6	50–70	27	
101	2F4/11		91.3	16	98.7	68.4	10.8	9	>90	95	
102	2A10/8	SWC7	1.2		5.7	3.3	5.9	1.8	negative	negative	
106	BL3F1	CD11/18	86.2	41.9	81.7	77.6	2.8	2.4	60–80	61	
112	1E12/11		97.5	41.1	5.1	9.8	3.6	1.9	negative	negative	>205
113	2A10/11		86.8	31.2	10.8	54.3	3.5	3.8	20–40	<2	150
114	3B11/11		93.9	47.7	7.3	10.3	3.2	2.8	negative	negative	>204
115	3F7/11		96.7	38.7	5.5	20.8	3.9	32.5	<10	<2	135
117	4E9/11		36.8		21.2	11.5	7.7	22	<5	<4	125–170
119	BL1H7		85.6	13.1	89.5	68.9	6.3	46.7	>90	>99	90–110
121	MIL2		79.1	38.6	92.7	53.2	13.5	3.9	82	66	50–55
122	MIL4		23.8	39.1	54.4	25.8	11.5	39	<10	33	
126	MIL10		31.8	40.5	97.8	3.9	16.8	34.7	<2	71	
133	PM16-6		67.9	35.8	83.5	43.2	5.6	64.5	50–70	95	
134	F3-9F2-E4		3.2		2.7	1.8	3.5	7.8	negative	negative	150
141	JM7B6		25.6		96.4	7.9	41.4	37.3	<5	95	30
143	ICRF44		10.2		33.4	8.3	10.1	19.7	<5	6	
144	TU66	CD39	8.8		3.5	3.3	4.8	11.5	negative	negative	
153	STH224		67.9		2.4	32.7	17.2	17.1	20–40	<3	
154	STH083		16.3		5.6	8.9	16.6	16.8	<10	<2	
157	STH208		56.8	30.8	42.3	43.7	5.1	56.6	>80	96	
158	STH277		48.1	33.6	69.5	48.6	6.3	54	>80	96	
159	STH241		91.2	39.7	92.1	66.1	4.3	8	>90	75	
160	STH226		3.9	40.8	93.4	25.1	3.9	4.7	50–70	53	
167	1030H-3-17		1.9		3.1	41.9	6.6	2.6	negative	negative	
168	1030H-3-17		1.9		3.1	41.9	6.6	2.6	negative	negative	
174	1038H-11-11		76.6		61.4	63.3	13.3	28.8	<5	<4	
186	C35	CD21	92.3		98.3	80.8	16.2	10.7	>80	93	

187	IC2F10		85.7	39	3.1	28.1	4	13.4	<5	<3	80
188	C25	CD11b	87.6	14.1	95.4	71.7	11.6	12.3	<80	92	160/95
190	FA2F12		7.7		12.1	8.2	10.9	5.6	negative	negative	
195	UCP2E1		93.8		98.8	68.7	15.8	25.7	50–70	75	
196	UCP1B2		21.8		29.6	6.8	12.5	35.8	negative	negative	
200	PM18-7	SWC	9	87.9	57.3	5.8	3.2	5.3	10.9	<5	4

[a] Pulmonary alveolar macrophage.
[b] Monocyte derived macrophages.
[c] Peripheral blood granulocytes.
[d] Peripheral blood monocytes.
[e] Peripheral blood lymphocytes.
[f] SWC3-positive cells from jejunum.
[g] Bone marrow monocytes.
[h] Bone marrow granulocytes.
[i] Molecular weight determined by immunoprecipitiation-SDS-PAGE during workshop analysis.

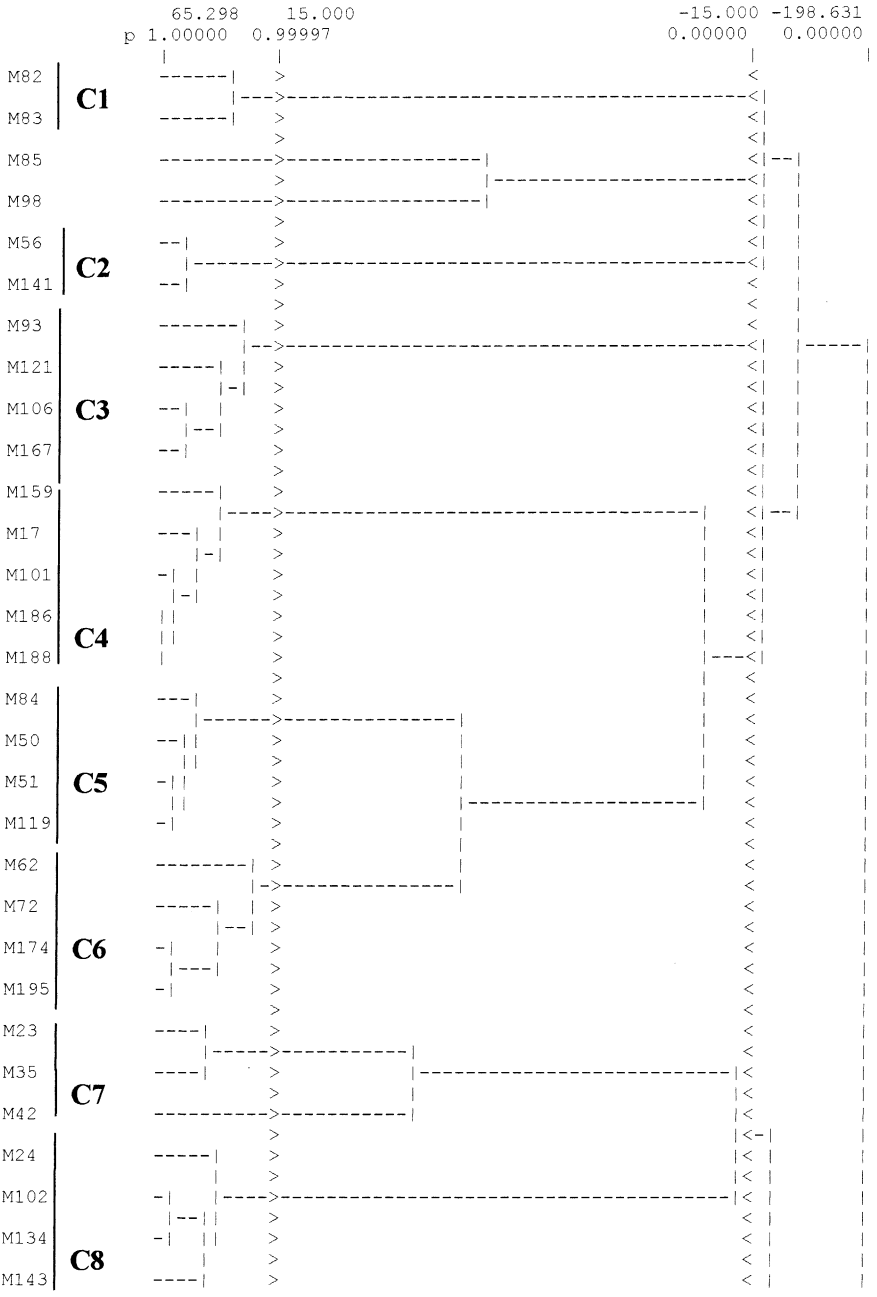

Fig. 1. Modified computer printout of cluster analysis of all mAb in the myeloid cell section, based on 65 individual data sets. The mAb are indicated by their workshop numbers; clusters are described with numbers : (A) C1–C8; (B) C9–C17.

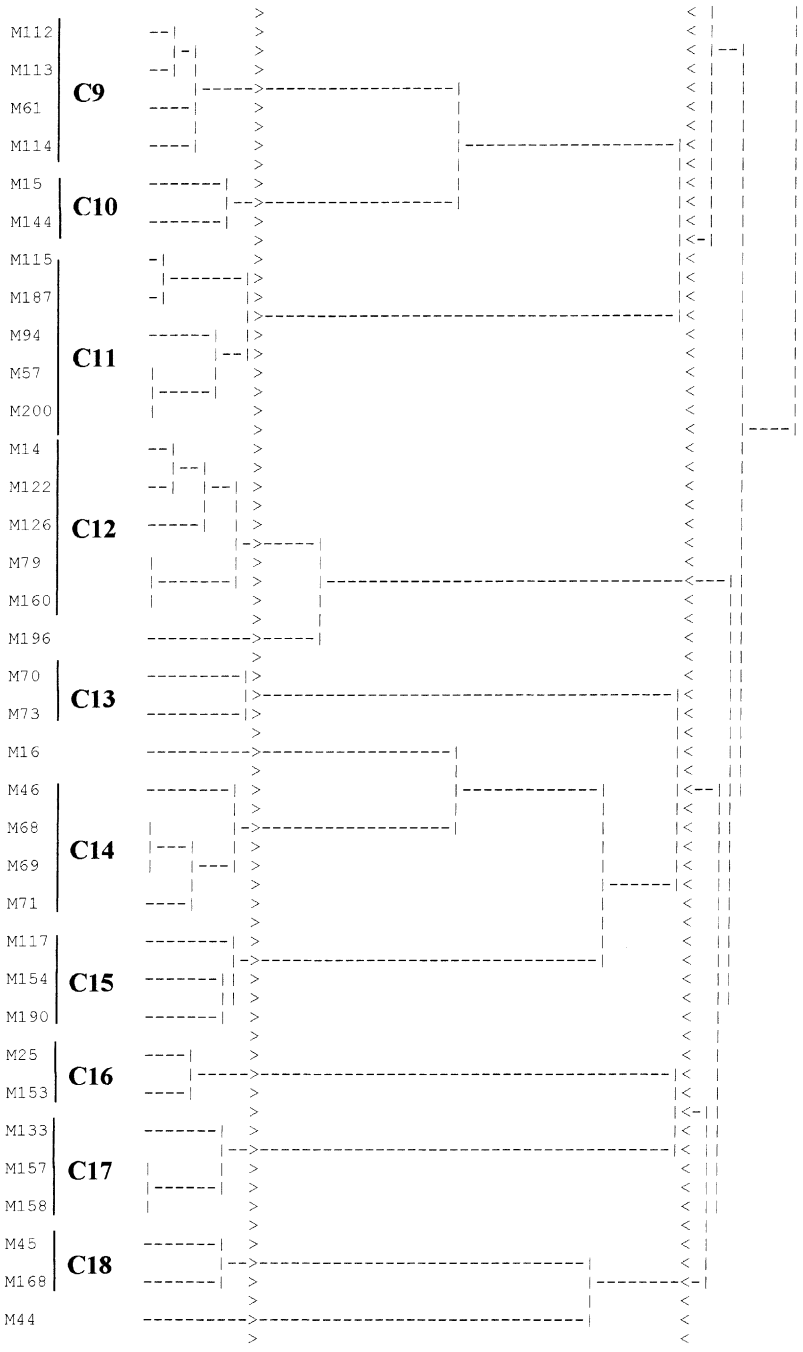

Fig. 1. (*Continued*).

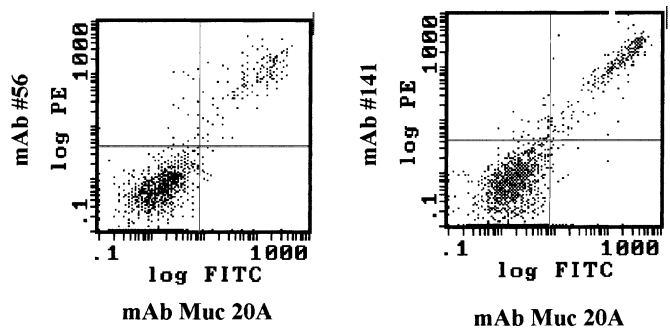

Fig. 2. Two-colour FCM profiles of mAbs no. 56 (MIL3) and no. 141 (JM7B6) and MUC 20A on PB mononuclear cells.

Cluster 3 consisted of four mAbs. The mAb 2C12/10 (no. 93) had a different binding pattern on the various cell populations than the rest of the mAb in this cluster and was not investigated further. The reactivity of MIL2 (no. 121) will be discussed later.

The mAbs BL3F1 (no. 106) and 1038H-3-16 (no. 167) clustered closely together and with mAbs C25 (no. 188) and 2F4/11 (no. 101) from C4 recognize a CD11 antigen as described by (Dominguez et al., 1998). C25 (no. 188), 2F4/11 (no. 101) and BL3F1 (no. 106) precipitated identical bands of approximately 155 kDa. Double labeling FCM analysis found that BL3F1 (no. 106), C25 (no. 188) and also 1038H-3-16 (no. 167) appear to recognize the same antigen (Dominguez et al., 1998). The fact that BL3F1 (no. 106) and 1038H-3-16 (no. 167) did not cluster closely with C25 (no. 188) and 2F4/11 (no. 101) demonstrates that care must be taken when attempting to interpret clustering analysis. Two colour FCM suggested that STH241 (no. 159) in C4 may be of SWC3 specificity (not shown), but no epitope analysis was carried out to support this finding. The last antibody in this cluster, G7 (no. 17) is known to be specific for CDI6 (Halloran et al., 1994).

3.4. Cluster C5

Cluster C5 is made up of four mAbs. Two of these, 74-22-15 (no. 50) and 6F3 (no. 51), were designated SWC3a in the First Swine CD Workshop. They bind the majority of monocytic cells and neutrophils. They also appear to bind to some cultured endothelial cells, an observation not reported previously for SWC3. BL1H7 (no. 119) appeared to have a similar staining pattern and reactivity to 74-22-15 (no. 50) and 6F3 (no. 51), with which it clustered closely. Two colour FCM (not shown) supported the SWC3 reactivity of BL1H7 (no. 119), justifying a SWC3 assignment of this antibody. Further work needs to be performed to determine if the epitope recognized is identical to 74-22-15 (no. 50) and 6F3 (no. 51).

PGBL21A (no. 84) exhibited a staining pattern similar to the antibodies recognizing CD11b (Dominguez et al., 1998). However, the sample volume was inadequate to confirm the CD11b specificity by IP or double labeling FCM. Antibody PGBL18A (no. 82) had a similar staining pattern to PGBL21A (no. 84) on myeloid cells, but clustered as C1 with

PGBL1A (no. 83) and showed no specificity for myelomonocytic cells, but rather recognized subsets of all the various cell types included in the first round clustering. In addition PGBL18A (no. 82) and PGBL21A (no. 84) appeared to recognize erythrocytes in the BM suggesting that these mAbs may not recognize CD11. Additional work needs to be performed to further identify the cell antigens recognized by these mAb.

3.5. Cluster C6

This cluster consists of a group of mAb exhibiting heterogenous reactivity to myelomonocytic cells. All mAb in this group appeared to recognize cultured endothelial cells. Mab no. 62 and no. 195 recognized a substantial proportion of BM cells, while mAb no. 72 and no. 174 did not. In the second Swine CD Workshop RHIA (no. 72) clustered closely to G7 (no. 17 in the current workshop) recognizing CD16 (Halloran et al., 1994). In the present workshop, G7 (no. 17) clustered in C4. RH1A (no. 72) bound strongly to macrophages and PB granulocytes and monocytes. It did not appear to recognize a specific myeloid cell type.

In the Second Swine CD Workshop, SwNL-552.2 (no. 62) clustered with the SWC3a mAb 74-22-15. In this workshop it clustered separately. SwNL-552.2 (no. 62) did not stain as intensely as the other anti-SWC3a mAbs, which may account for the difference in the percentages of cells stained and thus the separate clustering. Two colour FCM did not support SWC3 specificity for SwNL-552.2 (not shown) and SwNL-552.2 (no. 62) did not bind as many PAM as the other SWC3a mAb. This suggests the antibody does not recognize the same antigen. However, there was insufficient quantity of the mAb to perform further assays to confirm the specificity of SwNL-552.2 (no. 62). The remaining mAb in this cluster, 1038H-11-11 (no. 174) and UCP2E1 (no. 195) had different binding patterns. The differences in binding and clustering patterns observed with these three mAb suggest they are probably detecting different myeloid antigens.

3.6. Cluster C9

The mAb in this cluster bound strongly to macrophages and/or monocytes, and much less to any other cells used in the study including myeloid cells from PB or BM. Sanchez et al. (1999) found that 2A10/11 (no. 113) recognized a protein homologous to human CD163, a monocyte/macrophage differentiation marker with a MW of 150 under reducing conditions and MW of 120 kDa under non-reducing conditions (Sanchez et al., 1999). This workshop confirmed that Mab 2A10/11 (no. 113) as well as SwNL-517.2 (no. 61) bound specifically to monocytes and macrophages, and binding of SwNL-517.2 (no. 61) inhibited 2A10/11 (no. 113). However, some immunohistological studies of mucosal sites appeared to show differences between these two antibodies (not shown), therefore, some doubts remain and SwNL517.2 (no. 61) was provisionally assigned to wCD163. The remaining two mAb, 1E12/11 (no. 112) and 3B11/11 (no. 114) bound exclusively to macrophages in this study. Both antibodies recognize a molecule of MW of >204 kDa under reducing conditions, and of 190 kDa under non-reducing conditions.

3.7. Cluster C11

This cluster contains five mAb with ligands found predominantly on macrophages. Neither mAb bound significantly to any PB cells in this workshop. Two mAb, C4 (no. 57) and PM18-7 (no. 200) were previously identified as SWC9 in the Second Swine CD Workshop and precipitate two bands of 130 and >204 kDa. (Dominguez et al., 1998). These two mAb recognize mature macrophages and approximately 50% of thymocytes.

The remaining three mAb 5A6/8 (no. 94), 3F7/11 (no. 115) and IC2F10 (no. 187) did not recognize thymocytes, however, their ligand(s) were also detected on endothelial cells and endothelial cell lines. MAbs 3F7/11 (no. 115) and IC2F10 (no. 187) clustered closely together and also recognized the B cell line, L14. However, the molecular weights of the proteins differed, suggesting that while the two mAb detect similar populations of cells, they do not bind to the same antigens on the cells.

3.8. Cluster C12

TMG6-5 (no. 14) cross reacts with the anti-human CD11b mAb. In addition, TMG6-5 (no. 14) and MIL4 (no. 122) appear to recognize the same antigen. The reactivity of these two antibodies as well as other CD11 specific antibodies is discussed in detail in this issue by (Dominguez et al., 1998) MIL 10 (no. 126) differs from TMG6-5 (no. 14) and MIL4 (no. 122) in binding pattern and appears to strongly bind to PB granulocytes. PG68A (no. 79) and STH226 (no. 160) appeared to be have similar binding patterns, based on their reactivity with mature neutrophils and monocytes, but not eosinophils.

3.9. Cluster C17

All three mAb in this cluster, PM 16-6 (no. 133), STH208 (no. 157) and STH277 (no. 158) had also clustered together in the Second Swine CD Workshop. In both the current workshop and the Second Swine CD Workshop, it was determined that they appear to be non-specific for myeloid cells. These mAb may be recognizing a non-lineage specific antigen, the expression of which may be dependent on the cell activation status as demonstrated by the increased binding of the mAb with mitogen activated cells (data not shown). No further analysis was performed on any of these mAb.

3.10. CD14

The internal CD14 standard included in this workshop was biG10 (no. 16), an anti-human CD14 mAb. Previous workshops have established that biG10, together with several other anti-human CD14 mAbs including, TÜK4 and MY4, recognize porcine CD14 (Dominguez et al., 1998; Kielian et al., 1994). In addition to these established mAbs, several additional anti-human CD14 antibodies including MEM-18 have been reported to recognize procine CD14 (Schütt et al., 1995; M. Labeta, personal communication). However, although the workshop mAbs CAM36A (no. 85) and MIL2 (no. 121) were proposed to have CD14 reactivity by their donors, they failed to cluster, either with each other or with the internal CD14 standard biG10 (no. 16). Nevertheless,

double labeling analyses, competition studies and molecular weight analyses confirmed the specificity of both CAM36A (no. 85) and MIL2 (no. 121) for CD14.

MIL2 (no. 121) precipitated a molecule of approximately 50 kD, corresponding to the expected size of CDl4 (data not shown). No molecular weight data was available for CAM36A (no. 85). MIL2 (no. 121), but not CAM36A (no. 85), has been shown to cross

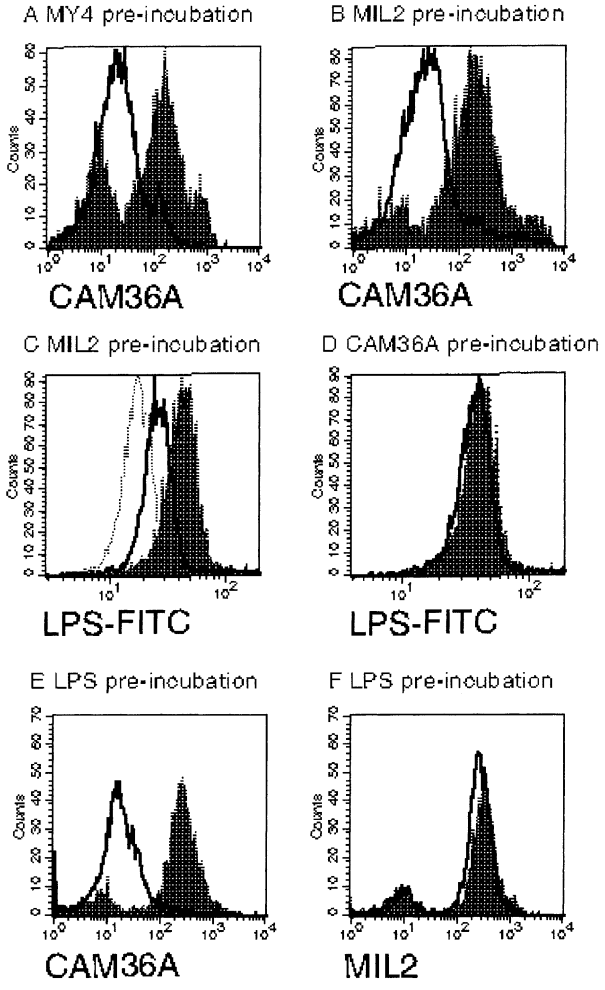

Fig. 3. Competition studies with anti-CD14 mAbs. PB leukocytes were gated electronically for granulocytes and monocytes based on typical scatter characterisitics. In A, cells were pre-incubated for 20 mm with MY4 (solid unfilled overlay), in B and D with no. 121 MIL2 (solid unfilled overlay) or MEM-l8 (D, dashed unfilled overlay), in C with no. 85 CAM36A (solid unfilled overlay), and in E and F with LPS (100 μg/ml, solid unfilled overlay). This was always followed by a wash step before immunofluorescence labelling with no. 85 CAM36A (A, B, E) no. 121 MIL2 (F) or LPS-FITC (C, D; 100 μg/ml, Sigma Chemicals, Buchs, Switzerland). The filled histograms represent the fluorescence obtained without pre-incubation with the competing reagent.

react with human monocytes and granulocytes in a pattern compatible with the distribution of human CDl4.

Both MIL2 (no. 121) and CAM36A (no. 85) demonstrated a CD14-like maturation-dependent expression on BM cells (Summerfield and McCullough, 1997). As shown in Fig. 3A, the binding of CAM36A (no. 85) to PB leukocytes was blocked by MY4. Similar results were obtained with TÜK-4 but not with MIL2 (data not shown). Although pre-incubation of the cells with MY4 did not reduce MIL2 (no. 121) binding, MIL2 was able to block CAM36A (Fig. 3B). Blocking experiments showed that pre-incubation of cells with MIL2 and MEM-18 blocked FITC-labeled LPS binding (Fig. 3D). CAM36A did not block LPS binding (Fig. 3 C). Conversely, pre-incubation of leucocytes with LPS efficiently reduced CAM36A reactivity (Fig. 3E). Interestingly, LPS pre-incubation had little influence on MIL2 binding (Fig. 3F).

Fig. 4 shows the binding of CAM36A (no. 85), MIL2 (no. 121), biG10 (no. 16) and TÜK4 on the different cell types of PB. Gating based on FS/SS characteristics showed that PMN express CD14 at low intensity and monocytes at high intensity (not shown).

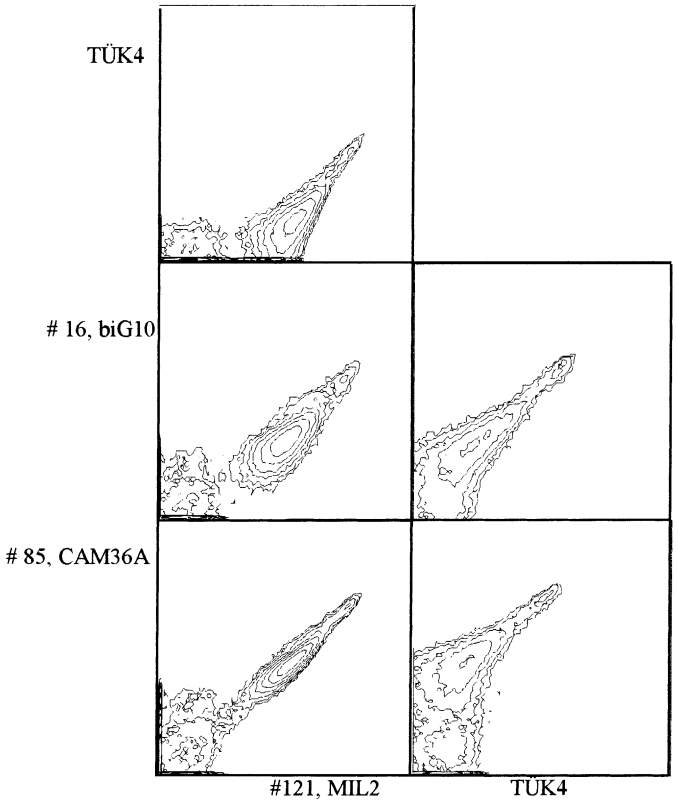

Fig. 4. Two-colour FCM profiles of mAbs no. 16 (biG10), no. 85 (CAM36A), no. 121 (MIL2) and TÜK4 on PB leucocytes.

These results are similar to the CD14 expression on human cells. The mean intensity of fluorescence (MIF) for biG10 and TÜK4 was considerably lower than for MIL2 and CAM36A. This resulted in PMN staining with biG10 and TÜK4 ranging from negative to low positive; consequently only 21% of the PMNs stained positive with biG10 (Table 2 and Fig. 3). In contrast, the increased intensity in staining by CAM36A and MIL2 resulted in 89 and 93%, respectively of the granulocytes appearing positive.

The mAb 4F10 (no. 98), although clustering close to CAM36A, appeared to have different reactivity on the various cell populations, as well as a MW (>21 kDa) not compatible with CD14 reactivity.

4. Summary and conclusion

The need to identify cells of the myelomonocytic lineage in swine has increased in importance due to the potential use of swine as xenotransplantation donors, large animal models for immunological research and the increasing number of pathogenic organisms that preferentially infect macrophages. The ability to identify specific populations and subpopulations of myelomonocytic cells will further our understanding of the pathogenesis of important swine diseases induced by African Swine Fever virus, classical swine fever (hog cholera) virus, porcine reproductive and respiratory syndrome virus Aujesky's disease virus and circovirus, all of which are known to infect macrophages.

The identification and specificity of 12 mAbs putatively recognizing three different CD11 molecules was determined is described by (Dominguez et al., 1998). These integrins are important in cell to cell and cell to matrix interactions. Characterization of these mAb will aid research projects thus increasing our knowledge of the interactions between cells.

In addition, mAb JM7B6 (no. 141) was assigned to SWC8, a pig specific cluster of determination established in the previous workshop with MIL3 (no. 56) and MUC20A. In that workshop it was determined that these antibodies recognize an epitope on all granulocytes, on B cells and on a subset of T lymphocytes, but not on resting PAM or monocytes. This workshop confirmed that the epitope recognized is not present on resting monocytic cells, but also showed that binding to both cell types increased following activation and culture. Cultured endothelial cells also express this ligand to varying degrees. Further work needs to be performed to characterize the molecules recognized by these mAbs.

Two new mAbs were assigned to porcine CD14. Epitope analysis gave no definitive results, as not all blocking combinations were possible due to a lack of directly conjugated antibodies, additionally, blocking frequently appeared due to steric hindrance rather than true epitope blocking. However, the work did show that the two new antibodies detect different epitopes of CD14. More than five distinct epitopes have been identified on human CD14, several of which can interfere with LPS binding, and the four cross-reactive antibodies used in this study were shown to recognize three different epitopes on human CD14 (Schütt et al., 1995). It was shown in this work that MIL2 (no. 121) and MFM-18, but not CAM36A (no. 85) could block LPS binding. Of interest for

using these antibodies as diagnostic reagents is the fact that the two groups of antibodies exhibit different binding affinities for granulocytes. Although all the CD14 specific mAb bind strongly to monocytes, biG10 and TÜK4 appear to bind granulocytes with very low affinity. In contrast, MIL2, CAM36A and My4 give clearly positive results on PMN and show even stronger labeling of monocytes. This difference in binding patterns was also noted in the myeloid section of the second workshop (Dominguez et al., 1998). A possible explanation may be that the epitope(s) recognized by the first group are less accessible on granulocytes than on monocytes.

Characterization of mAb recognizing myelomonocytic cells remains difficult due to the many differing surface molecules which vary in their expression levels. Interpretation of the results was further complicated by the diverse population of cell types in the samples collected. The binding of the mAb to the various cell populations often differed significantly between laboratories making interpretation of the results difficult. Potential sources of disparity in results obtained between laboratories may be due to alteration of the surface antigens during activation associated with the health status of the animal and/or the collection and isolation procedures used to obtain the cells. Levels of competing target antigens present in serum, such as soluble CD14 may also add to between and within laboratory variation. These problems may explain such clustering anomalies as the ones experienced with the anti-CD14 antibodies. Immunohistochemistry and other assays which determine the location and identity of the molecules recognized by the mAb as well as their size, function and sequence homologies is required to complete the identification of the various myelomonocytic populations.

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